

REMARKS

1. Claim Amendments

We assume that claim 1, and all claims dependent thereon, are now in condition for allowance, unless there is a non-art issue specific to one of the dependent claims which is not cured by the amendment of the base claim.

Claim 1 has been amended to incorporate the limitation of claim 56, of at least 90% identity to SEQ ID NO:2. Consequently, claim 56 has been cancelled as redundant.

Claims 4, 5, 56-58, 64, and 70-72 were deemed allowable (OA p. 11), but objected to merely as dependent on a rejected base claim.

Claim 64 is amended as recommended by the Examiner (OA p. 11). Claim 65 has been amended to require that the isolated polynucleotide (c) is one "comprising a nucleotide sequence encoding a multisubstrate deoxribonucleoside kinase". This functional limitation is copied from claim 1. Claims 73-75 have been cancelled.

Claims 59 ad 61 have been amended to explicitly list the non-conserved residues, while claims 60 and 62 have been cancelled.

2. Enablement

2.1. Claims 65-68 and 73-75 were rejected (OA pp. 5-6) were rejected solely because we deleted the functional limitation that the encoded polypeptide has dNK activity. We have amended claim 65 to require the same functionality set forth in claim 1. This should overcome the rejection vis-a-vis claim 65 and dependent claims 66-68. Claims 73-75 have been cancelled.

2.2. Claims 1-3, 6-7, 9, 16, 19, 20, 22, 55 and 63 were rejected because the disclosure was deemed inadequate to support 85% identity at the amino acid level or (per claim 69) 90% identity at the polynucleotide level. The Examiner has conceded enablement for 90% identity at the amino acid level (OA p. 8), per claim 56. The main claim has been so amended, and hence this

rejection should be withdrawn. (The amendment is without prejudice to pursuit of 85% identity claims in a continuation application.)

### 3. Definiteness

3.1. Claims 2-3 stand rejected for alleged indefiniteness. The examiner asserts that it is unclear "what property is being compared and how the comparison is a limitation of the claim". These are original claims of the application and are therefore part of the disclosure. They are also based on page 8, lines 15-18, which expresses particular interest in the effect of the kinase on the analogues gemcitabine and araC.

The property being compared is the IC50 of at least one nucleoside analogue (e.g., gemcitabine or araC) as a result of exposure to the phosphorylation activity of the claimed kinase. However, the claim looks not merely at the absolute effect of the claimed kinase on the analogue's IC50, but rather the effect of the claimed kinase relative to that of HSV TK1 kinase. So, if for example the HSV TK1 kinase reduced the IC50 of a particular analogue by two-fold, the claimed kinase would have to reduce it by at least eight-fold (four times two).

The comparison limits the claim because claim 1 may cover DNAs which encode mutants whose superiority to HSV TK1 kinase is less pronounced than that of the reference mosquito kinase, such that they don't satisfy the "at least four-fold" limitation of the claim.

"IC50" is a standard abbreviation meaning "50% inhibitory concentration", i.e., the concentration of an inhibitor needed to inhibit half of the reference level of activity of a biological process or component.

Claim 2, as written, did not specify what was inhibited. However, we direct the examiner's attention to the following teachings:

"the invention provides ... method of inhibiting unwanted [sic, "unwanted"] cell growth...."  
(P1, L14-17)

"the enzyme acts on the substrate to generate a substance toxic to the targeted tumor cells".  
(P2, L11-12)

"the invention provides method of sensitising target cells to prodrugs"  
(P4, L38-39)

"methods of inhibiting pathogenic agents in warm-blooded animals"  
(P5, L5-6)

"Once prodrugs are converted into a potent cytotoxic metabolite they inhibit or disrupt DNA synthesis. The treated cells subsequently die via necrotic or apoptotic pathways".  
(P6, L27-29)

"The dNK kinase enzyme of the invention is particularly useful for the treatment of abnormal cell growth by activating nucleoside analogs, in particular gemcitabine".  
(P6, L36-38).

We have accordingly amended claim 2 to specify that the IC50 is a measure of the inhibition of cell growth. The "unwanted cells" could be cancer cells, see P17, L15 to P18, L16,, or a viral-infected cell, See P8, L20-21. Since it is perhaps awkward to speak of 50% of a single cell, we have converted "cell" to "cells".

The unphosphorylated nucleoside analogue is a prodrug, which is converted to active form by a kinase such as the claimed kinase. Thus, the purport of claims 2-3 is to quantify the efficiency of the claimed kinase in phosphorylating an unphosphorylated nucleoside kinase and thereby converting it to the active form in which it causes inhibition of cell growth (e.g., by inhibition of DNA synthesis) and ultimately cell death.

More particularly, the claims quantify the efficiency of the claimed kinase **relative to HSV TK1 kinase** in phosphorylating the nucleoside analogue and thus in reducing the dose of the unphosphorylated nucleoside analogue needed to achieve a 50% reduction in cell growth. We have amended claim 2 to make this clear by moving the limitation concerning comparison to HSV TK1

kinase, and making specific reference to the reduction in cell growth as a result of the phosphorylation of the analogue.

Claim 3 has been cancelled; it was limited to mutants of the mosquito dNK and we see no need to claim that separately.

In addition we add new claim 76. While in claim 2, the kinase's activity is quantified as an effect on the toxicity of the nucleoside analogue measured as an IC50 for inhibition of cell growth, in claim 76 that toxicity is expressed directly as an LD100, that is, the dose of the unphosphorylated analogue which, as a result of its phosphorylation by the kinase, results in the killing of 100% of the cells. There is, of course, a direct correlation between the IC50 against cell growth, and the LD100.

Claim 76 is based on example 3, table 5, wherein applicants state the "LD100" of the (unphosphorylated) nucleoside analogues dFdC and ara-C in four contexts: KY895 cells only, cells transformed with the empty vector pGEX-2T, cells transformed with the mosquito kinase of vector PZG318, and cells transformed with the HSV TK1 kinase of vector pGEX-2T-HSV1-TK.

The "at least ten-fold" limitation of claim 76 is based on page 29, line 26, which notes that the LD100 for ara-C, in the mosquito kinase context, was at least ten fold lower than that for ara-C in the HSV TK-1 kinase context.

3.2. Claim 59 is questioned because it does not recite the amino acids which are "non-conserved". Contrary to the Examiner's suggestion, these residues can be identified without consideration of the "scientific literature". All of the residues of SEQ ID NO:2 are classified as conserved, semi-conserved, or non-conserved by Fig. 1. If the Ae-DNK residue is shown as white on black, it is conserved; if it is black against a dotted background, it is semi-conserved; and it is normal text, it is non-conserved. The list of conserved and semi-conserved residues in claim 59 was simply extracted, by a purely clerical process, from Fig. 1. In like manner one can extract a list of

non-conserved residues: A3, G6, P7, V12, A13, etc. Claim 59 has been suitably amended.

3.3. The same argument, and solution, applies to claim 61. However, it was applicant's intent that claim 61 be narrower than claim 59 (allow mutation only at the "non-conserved" residues, rather than at "non-conserved" or "semi-conserved" residues), and claim 61 inadvertently just categorized the residues without limiting the substitutions. Claim 61 is now amended as intended.

3.4. Claims 60 and 62 have been cancelled.

#### 4. Restriction

The restriction was based on a posteriori lack of unity. Since the Examiner deemed claim 56 to be allowable, and claim 1 has now been amended to incorporate the limitation of claim 56, the argument of a posteriori lack of unity no longer applies and the withdrawn claims should be rejoined.

Because the case is under final rejection, we don't consider ourselves to be at liberty to amend the withdrawn claims. However, we would be happy to consider an examiner's amendment to conform them to the instant group 1 claims.

Respectfully submitted,

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